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# DETERMINATION OF METSULFURON METHYL RESIDUES IN BOVINE SAMPLES

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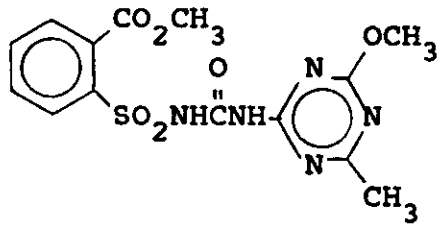
## ABSTRACT

A procedure has been developed for the determination of metsulfuron methyl, methyl 2-[[(methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]aminosulfonyl] benzoate, residues in bovine cream, fat, feces, milk, tissues, and urine. Metsulfuron methyl was extracted from cream, milk, and urine with toluene. It was extracted from feces and tissues with an acetone-aqueous mixture and from fat with acetonitrile. The samples were then cleaned up by methylene chloride washes of a basic solution and on a silica Bond Elut® cartridge.

Final determination was by normal phase HPLC with a photoconductivity detector. Recoveries for 99 fortified samples averaged 90% with a standard deviation of 11%.

## INTRODUCTION

Methyl 2-[[(methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl]aminosulfonyl ] benzoate, (metsulfuron methyl), with the structure,



DPX-T6376

is a Du Pont experimental sulfonylurea herbicide. Metsulfuron methyl, formerly known as DPX-T6376, is effective in controlling a variety of weeds in cereal grain fields. Since cereal plants may be fed to cows, methods have been developed to determine metsulfuron methyl residues in bovine samples.

Metsulfuron methyl residues in feces and tissues were determined by the procedure developed for the analysis of crop samples (1). Fat samples were also analyzed by the same procedure except they were extracted with acetonitrile rather than an acetone-buffer solution.

Metsulfuron methyl was extracted from cream, milk, and urine with toluene after the sample had been acidified with HCl. The samples were then cleaned up on silica Bond Elut®

cartridges. An additional wash step was added just before the final determination for cream samples. Acetonitrile solutions of the samples were washed several times with hexane to remove oils.

Metsulfuron methyl residues in all the samples were determined by normal phase HPLC using a photoconductivity detector. Recoveries for 99 bovine samples fortified with metsulfuron methyl averaged 90% with a standard deviation of 11%.

## Analysis Procedures

### Equipment and Reagents

A Du Pont Model 8800 Liquid Chromatograph was used for analysis of samples. The chromatograph consisted of a microprocessor controller, Model 870 pump, column compartment, and a data system. The column compartment was fitted with a Model CU-6-UHPa-N60 Valco valve. The column was a Zorbax® SIL, 4.6 mm i.d. x 25 cm column. The liquid chromatograph, valve, and column were purchased from Analytical Instruments Division, E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware. The detector was a Tracor Model 965 Photoconductivity detector purchased from Tracor Instruments, Austin, Texas.

For homogenization and extraction of samples, a Tekmar Tissumizer®, Model SDT-1810, with a Model SDT-182 EN shaft and generator was employed. The Tissumizer® was purchased from

Tekmar Company, Cincinnati, Ohio. For concentration of samples, a vacuum rotary evaporator with a 45°C water bath and pear-shaped flasks, No. K-608700, purchased from Kontes, Vineland, New Jersey was used. An N-EVAP®, Organomation Assoc., Worcester, Massachusetts (water bath at room temperature) was used for concentrating the samples to final dryness under nitrogen.

A Vortex-Genie® mixer was used for mixing of samples in centrifuge tubes. An International Equipment Company Model K centrifuge was used to centrifuge samples. The centrifuge was fitted to hold either six 250 mL centrifuge bottles or sixteen 50 mL centrifuge tubes. A Guth® Universal wash bottle was used to rinse off the Tekmar Tissumizer®. The Vortex-Genie®, centrifuge and wash bottle were all purchased from Fisher Scientific, Pittsburgh, Pennsylvania.

A Millipore® all glass filter apparatus (No. XX15 04700) with a 0.5 um Teflon® filter (No. FHUP 04700) was used to filter solvents. Millipore® Millex®-SR disposable filters were used to filter samples. The 25 mm diameter filter unit contained a 0.5 um Teflon® filter. All Millipore® equipment was purchased from Millipore Corporation, Bedford, Massachusetts.

Silica and [redacted] cartridges were used for cleanup of samples. Each cartridge contained 500 mg of sorbent packed in a [redacted] disposable cartridge. The cartridges were fitted with either 15 mL or 75 mL reservoirs. A Vac Elut®

vacuum manifold was used to pull solvents through the cartridges. The cartridges, reservoirs, and manifold were all purchased from Analytichem International, Harbor City, California.

The reference standard of metsulfuron methyl was obtained from the Agricultural Products Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware. Brockman Activity I basic alumina was used to dry the mobile phase. The alumina (No. A-941) was purchased from Fisher Scientific. All solvents were UV, distilled-in-glass grade obtained from Burdick and Jackson, Laboratories, Muskegon, Michigan. The [redacted] (Type H1) was obtained from Sigma Chemical Company, St. Louis, Missouri. All other chemicals were ACS reagent grade obtained from Fisher Scientific.

Milk

Samples of milk (5.0 gram) were weighted into 50 mL glass-stoppered centrifuge tubes. To each sample was added 5.0 mL of deionized water, 1.0 mL of 10% HCl, and 15 mL of toluene. The tubes were then shaken for a minute and centrifuged at 2000 rpm for 10 minutes. While the samples were being centrifuged, a silica Bond Elut® cartridge for each sample was rinsed with 10 mL of 2-propanol followed by 10 mL of toluene.

The top toluene layer in each centrifuge tube was removed and transferred to a Bond Elut® cartridge using a glass syringe. Each sample was extracted two more times with 10 mL portions of

toluene as just described. The toluene was removed as before, run through the Bond Elut® cartridge and discarded. Each cartridge was then rinsed with 10 mL of toluene which was also discarded. Metsulfuron methyl was eluted from each cartridge with 5 mL of HPLC mobile phase (Table 1) which was collected in a 13 mL glass-stoppered centrifuge tube. The solvent was removed by evaporation under dry nitrogen, and the samples saved for chromatographic analysis.

Skim Milk

To analyze skim milk, two changes were made to the milk procedure. First, 10 mL of deionized water was added to 2.0 g of skim milk. Second, the samples were ultrasonicated for 2 minutes before they were centrifuged. If the emulsion did not break, the sample was ultrasonically agitated and centrifuged again.

Cream

To analyze cream, the milk procedure was used with the following changes: First, 10 mL of deionized water was added to each sample rather than 5 mL, and 15 mL of toluene was used for all extractions. Second, an extra cleanup step was necessary. After the effluent from the silica Bond Elut® cartridge had been evaporated to dryness, 5 mL of acetonitrile was added to each 13 mL centrifuge tube. Each sample was then washed with 3 x 4 mL portions of hexane. For each wash, the hexane and acetonitrile

were vortex mixed. The top hexane layer was removed and discarded using a glass syringe. After the third wash, the acetonitrile phase was concentrated to dryness under nitrogen evaporation and saved for HPLC analysis.

Urine

To analyze urine, the milk procedure was used with the following changes. Rather than adding 1.0 mL of 10% HCl to each sample, the pH was adjusted to 5.0 using 10% HCl and a pH meter. Four 15 mL toluene extractions of each urine sample were made and all toluene extracts were eluted through the silica Bond Elut© cartridge.

Tissue

The lean meat samples were ground twice with a meat grinder before they were sampled. Liver and kidney samples were ground once with a meat grinder.

Tissue samples (25 g) were placed in 250 mL glass centrifuge bottles and extracting solvent (80 mL) added to each bottle. The extracting solvent was made by mixing 800 mL of acetone with 200 mL of buffer B (Table 2), made by adding 1.64 g of sodium acetate and 1.0 mL of glacial acetic acid to 2.0 liters of deionized water.

Each sample, in a centrifuge bottle, was homogenized for 1 minute with the Tekmar Tissumizer®. After blending each sample, the shaft and generator were rinsed with a small portion (~10 mL) of extracting solvent from a Guth® wash bottle and the rinse added to the sample bottle. Any tissue caught in the generator was removed with tweezers and returned to the sample bottle. The samples were then centrifuged at 2000 rpm for 5 minutes. The liquid for each sample was decanted through a glass wool plug in a funnel and collected in a 250 mL volumetric flask. The extraction was repeated two more times with 70 mL of extracting solvent each time. After the third extract had been added to each volumetric flask, the samples were made to volume with extracting solvent.

A 25 mL aliquot of each sample was pipetted into a 250 mL separatory funnel which contained 100 mL of buffer A, (Table 2) made by dissolving 16.8 grams of sodium bicarbonate and 21.1 grams of sodium carbonate in 2.0 liters of deionized water. Each sample was then washed with 3 x 50 mL of methylene chloride by shaking for 1.0 minute each time. The bottom methylene chloride phase was removed each time and discarded. After the third wash, the aqueousphase for each sample was transferred to a 250 mL beaker. The pH was adjusted to 3.5 using a pH meter and 10% hydrochloric acid. Once the samples had been acidified, the next extraction step was carried out immediately.

The acidified samples were transferred back to the 250 mL separatory funnels and 50 mL of toluene added to each beaker. After swirling, the toluene rinse for each sample was added to the separatory funnel. Each sample was shaken for 1.0 minute, the bottom aqueous layer transferred to a 250 mL beaker, and the toluene then drained into a 250 mL glass centrifuge bottle. The aqueous solution in each beaker was poured back into the separatory funnel and extracted twice more with 50 mL of toluene each time. These extracts were combined with the first extract in the centrifuge bottle. The samples were centrifuged at 2000 rpm for 10 minutes.

Silica Bond Elut® cartridges fitted with 75 mL reservoirs were each rinsed with 10 mL of 2-propanol followed by 10 mL of toluene. The toluene layer in each centrifuge bottle was passed through a Bond Elut® cartridge, thus trapping the metsulfuron methyl on the silica. Care was taken to avoid removing any aqueous buffer from the centrifuge bottle. Additional toluene (25 mL) was added to each centrifuge bottle and the bottles were centrifuged for 10 minutes as before. After centrifuging, the toluene was removed and eluted through the cartridges. Each cartridge was then rinsed with 10 mL of toluene. Metsulfuron methyl was eluted from each cartridge with 5 mL of HPLC mobile phase (see Table 1), and collected in a 13 mL glass-stoppered centrifuge tube. The samples were concentrated to dryness with nitrogen and stored for HPLC quantitation.

Fat

Fat samples were ground with a meat grinder while frozen to break up the connective tissue. A 25 gram portion of each sample was placed in a 250 mL glasss centrifuge bottle. Hexane (60 mL) was added to the fat and each sample was homogenized with a Tekmar Tissumizer® until the fat was dissolved. Acetonitrile (75 mL) was added and each sample was homogenized with the Tissumizer® for 1.0 minute. The samples were then centrifuged 5 minutes, and the bottom acetonitrile layer for each sample transferred, using a 50 mL glass syringe, to a 250 mL volumetric flask. Two more acetonitrile:hexane (60 mL:10 mL) extractions were made and the extracts were added to the volumetric flask. After the third extraction, each volumetric flask was made to volume with acetonitrile. A 25 mL aliquot of each sample was added to 100 mL of buffer B in a 250 mL separatory funnel. The rest of the procedure was the same as for tissue.

Feces

The tissue procedure was followed except that an additional C-2 Bond Elut® cartridge cleanup step was added at the end. For each sample, a C-2 cartridge was rinsed with 10 mL of methanol followed by 10 mL of a 90:10 (buffer C:methanol) solution (Table 2), made by dissolving 1.38 g of sodium phosphate monobasic in 500 mL of deionized water. The pH was then adjusted to 3.0 using 10% $H_3PO_4$.

After each sample had been eluted from the silica Bond Elut® cartridge, it was evaporated to dryness with dry nitrogen. Each sample was then redissolved in 1.0 mL of methanol by ultrasonic agitation. Buffer C (9.0 mL) was added to each tube and vortex mixed. The sample mixture was then eluted through the prerinsed C-2 Bond Elut® cartridge. The transfer step was repeated with the same volumes of methanol and buffer C and this rinse eluted through the C-2 Bond Elut® cartridge. Each cartridge was next rinsed with 5 mL of deionized water. Metsulfuron methyl was eluted from each cartridge with 4 mL of acetonitrile which was collected in a 13 mL glass-stoppered centrifuge tube. The solvent was evaporated with dry nitrogen and the samples saved for HPLC analysis.

HPLC Analysis

A Du Pont Model 8800 HPLC was used for quantitation of metsulfuron methyl residues. A Tracor Model 965 photoconductivity detector was used because of its sensitivity and selectivity for metsulfuron methyl. A mercury lamp was used for photo-ionization of the sample. The ion exchange resin tube and pump were removed from the detector since they were not needed. The detector was further modified by placing a Nupro® metering valve (No. SS-25A-TFE) in the exit line from the reference conductivity cell. This was then adjusted to equalize the flow through the reference and analytical conductivity cells.

The HPLC mobile phase (Table 1) was made by mixing 690 mL of cyclohexane, 195 mL of 2-propanol, and 115 mL of methanol in a 2.0 liter beaker. One hundred grams of Brockman Activity I basic aluminia was added. After stirring with a magnetic stirring bar for 30 minutes, the mobile phase was filtered with a Millipore® all glass filter apparatus. To the filtered solution was added 3.0 mL of glacial acetic acid and 100 µL of deionized water. This was stirred for 10 minutes with a magnetic stirring bar.

A special solution (Solution A, Table 1) was used to dissolve the samples and standards. This solution was prepared the same as the mobile phase except the glacial acetic acid and deionized water were not added.

A column conditioning solution (Table 1) was made by mixing together 400 mL of 2-propanol, 400 mL of methanol, 200 mL of glacial acetic acid, and 40 mL of deionized water. This solution was also filtered with the Millipore® filter apparatus. New Zorbax® SIL HPLC columns were conditioned with conditioning solvent for 4 hours at 0.7 mL/min, then equilibrated with mobile phase for 3 hours at the same flow rate. The conditioning procedure was also used to clean columns which lost efficiency from contamination.

For analysis of samples, the HPLC flow rate was set at 1.0 mL/min and the oven temperature at 35°C. An injection

volume of 10 µL was used. The detector range was set to 1 and the recorder attentuator was set as needed.

A 100 µg/mL stock solution of metsulfuron methyl was made by dissolving 10 mg reference standard in 100 mL of ethyl acetate. Dilutions at 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, and 10.0 µg/mL in ethyl acetate were made for sample fortification.

A 1.0 µg/mL standard in Solution A was made by pipetting 1.0 mL of the 100 µg/mL stock into a 100 mL volumetric flask. The ethyl acetate was removed with dry nitrogen and the volumetric flask made to volume with Solution A. Working HPLC standards in Solution A at 0.25, 0.20, 0.15, 0.10, 0.05, 0.02, and 0.01 µg/mL were made in 10 mL volumetric flasks from the 1.0 µg/mL standard. These standards were prepared fresh weekly.

Each sample was dissolved in an appropriate volume of Solution A (Table 3 lists the volumes). To ensure complete dissolution, each sample was ultrasonically mixed, vortex mixed, and filtered with a Millex-SR® filter. Filtered samples were injected in the HPLC interspersed with standards. For any samples outside of the calibration range, an aliquot was diluted with Solution A and then reinjected.

Calculations

The sensitivity (S) for each standard in (mm-mL)/ng units was calculated by the equation.

$$S = \frac{P_s}{C_s} \quad \frac{1\ \mu g}{1000\ ng} \quad A \qquad (1)$$

$P_s$ is the peak height in millimeters, $C_s$ is the concentration of the standard in µg/mL, and A is the attentuation. The average sensitivity, $S_a$, was calculated and used for calculation of sample concentrations.

The sample concentration, C, in ng/g units (ppb) was calculated using the equation.

$$C = \frac{(P)\ (A)\ (F)\ (V)\ (D)}{S_a \quad W} \qquad W$$

P is the sample peak height in millimeters, F is the aliquot factor for samples which were aliquoted during the procedure, and V is the sample volume. D is the dilution factor for samples which were diluted for HPLC analysis and W is the sample weight in grams. These different factors are summarized in Table 3 for the different sample types.

## RESULTS AND DISCUSSION

Recovery data for cream, feces, kidney, lean meat, liver, milk, skim milk, subcutaneous fat, and urine are in table 4. Recoveries for all 99 samples fortified with metsulfuron methyl ranged from 54% to 138% and averaged 90% with a standard deviation of 11%. Chromatograms of a control milk sample, the same sample fortified with 0.01 ppm metsulfuron methyl, and a sample from a cow treated with 20 ppm metsulfuron methyl are shown in figure 1.

Chromatograms of a control skim milk sample, the same sample fortified with 0.025 ppm metsulfuron methyl, and a sample from a cow treated with 100 ppm metsulfuron methyl are shown in figure 2. Chromatograms of a control cream sample, the same sample fortified with 0.02 ppm metsulfuron methyl, and a sample from a cow treated with 20 ppm metsulfuron methyl are shown in figure 3.

Chromatograms of a control subcutaneous fat sample, the same sample fortified with 0.01 ppm metsulfuron methyl, and a sample from a cow treated with 100 ppm metsulfuron methyl are shown in figure 4. Chromatograms of a control lean meat sample, the same sample fortified with 0.01 ppm metsulfuron methyl, and a sample from a cow treated with 100 ppm metsulfuron methyl are shown in figure 5.

Chromatograms of a control liver sample, the same sample fortified with 0.01 ppm metsulfuron methyl, and a sample from a cow treated with 100 ppm metsulfuron methyl are shown in figure 6. Chromatograms of a control kidney sample, the same sample fortified with 0.01 ppm metsulfuron methyl, and a sample from a cow treated with 100 ppm metsulfuron methyl are shown in figure 7.

Chromatograms of a control urine sample, the same sample fortified with 2.0 ppm metsulfuron methyl, and a sample from a cow treated with 20 ppm metsulfuron methyl are shown in figure 8. Chromatograms of a control feces sample, the same sample fortified with 0.01 ppm metsulfuron methyl, and a sample from a cow treated with 5 ppm metsulfuron methyl are shown in figure 9.

## REFERENCES

1. L. W. Hershberger, "Determination Of Residues Of Metsulfuron Methyl In Crops By Liquid Chromatography;" AMR-104-84 revised November 26, 1984; Agricultural Products Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware.

TABLE 1

CHROMATOGRAPHIC SOLUTIONS

| Solvent | Mobile Phase | Solution A | Cleaning Solution |
|---|---|---|---|
| Cyclohexane | 690 mL | 690 mL | --- |
| Methanol | 195 mL | 195 mL | 400 mL |
| 2-Propanol | 115 mL | 115 mL | 400 mL |
| Glacial Acetic Acid | 3 mL | --- | 200 mL |
| Deionized $H_2O$ | 100 µL | --- | 40 mL |

## TABLE 2

## COMPOSITION OF BUFFERS

| | Buffer A | Buffer B | Buffer C |
|---|---|---|---|
| Deionized Water | 2000 mL | 2000 mL | 500 mL |
| Sodium Acetate | --- | 1.64 g | --- |
| Glacial Acetic Acid | --- | 1.0 mL | --- |
| Sodium Bicarbonate | 16.8 g | --- | --- |
| Sodium Carbonate | 21.1 g | --- | --- |
| Sodium Phosphate Monobasic | --- | --- | 1.38 g |

## TABLE 3

## FACTORS FOR EQUATION 2

| Sample Type | Final Volume (V) | Aliquot Factor (F) | Sample Weight (W) |
|---|---|---|---|
| Milk | 2.0 mL | 1 | 5.0 g |
| Skim Milk | 2.0 mL | 1 | 5.0 g |
| Cream | 2.0 mL | 1 | 5.0 g |
| Urine | 10.0 mL | 1 | 5.0 g |
| Tissue | 1.0 mL | 10 | 25.0 g |
| Fat | 1.0 mL | 10 | 25.0 g |
| Feces | 1.0 mL | 10 | 25.0 g |

TABLE 4

METSULFURON METHYL RECOVERIES FROM FORTIFIED SAMPLES

| Substrate | Fortification Range | Number of Samples | Recovery Range | Average Recovery | Standard Deviation |
|---|---|---|---|---|---|
| Cream | 10-100 ppb | 4 | 79-90% | 86% | 5% |
| Feces | 0.01-4.0 ppm | 3 | 68-82% | 78% | 7% |
| Kidney | 10-50 ppb | 4 | 73-138% | 96% | 29% |
| Lean Meat | 10-50 ppb | 6 | 69-99% | 83% | 11% |
| Liver | 10-50 ppb | 4 | 76-93% | 83% | 7% |
| Milk | 10-200 ppb | 55 | 76-118% | 92% | 8% |
| Skim Milk | 25-500 ppb | 4 | 76-90% | 84% | 6% |
| Subcutaneous Fat | 10-50 ppb | 4 | 54-85% | 73% | 15% |
| Urine | 0.1-40 ppm | 15 | 78-118% | 94% | 11% |

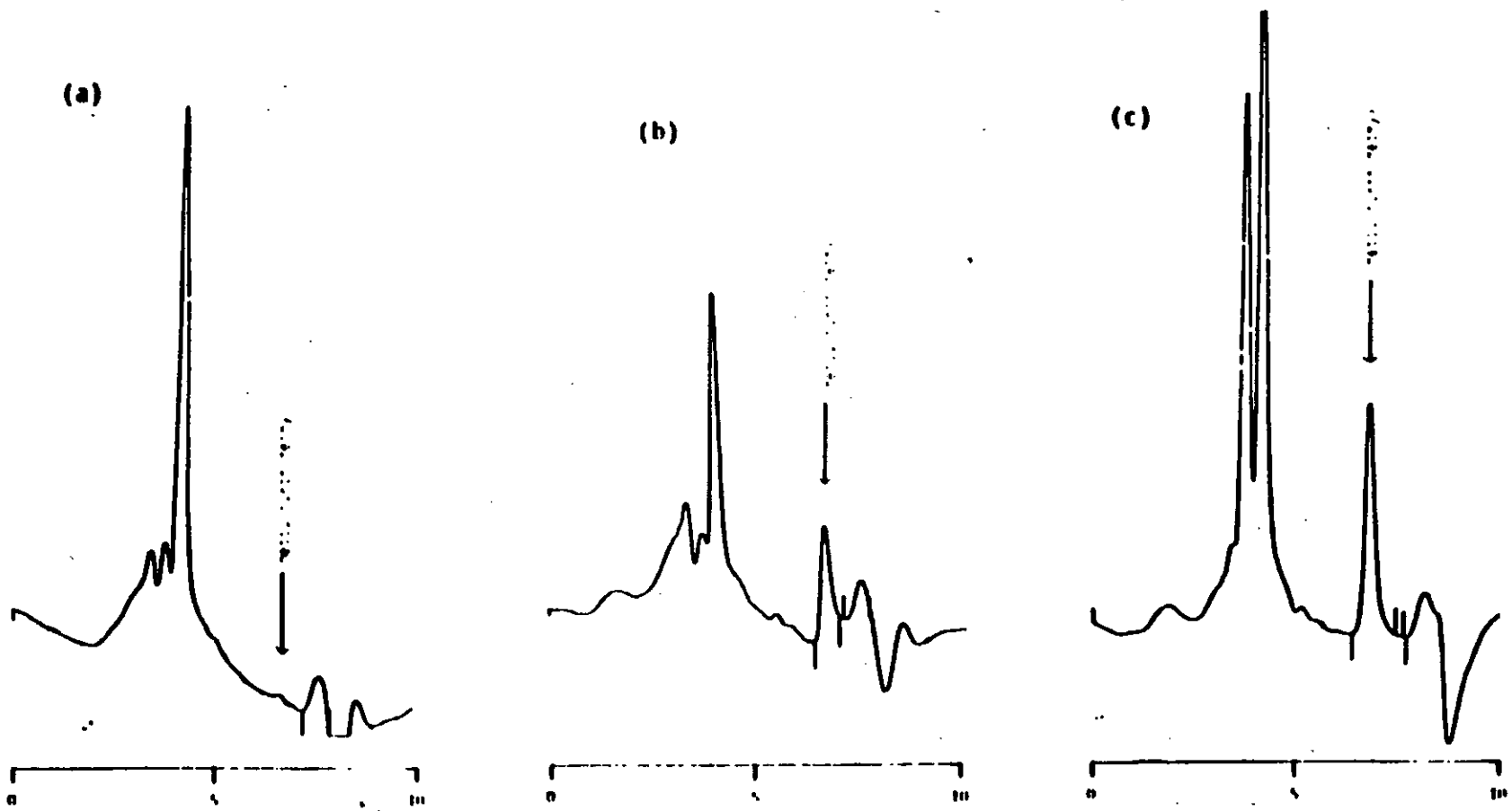



Figure 1. These are chromatograms of composited milk samples (a) a control cow, (b) the same control fortified at 0.01 ppm with metsulfuron methyl, and (c) a 20 ppm treatment rate cow. The recovery for the fortified milk is 98% and the residue level for the treated cow is 20 ppb. All chromatograms are at an attentuation of 1.

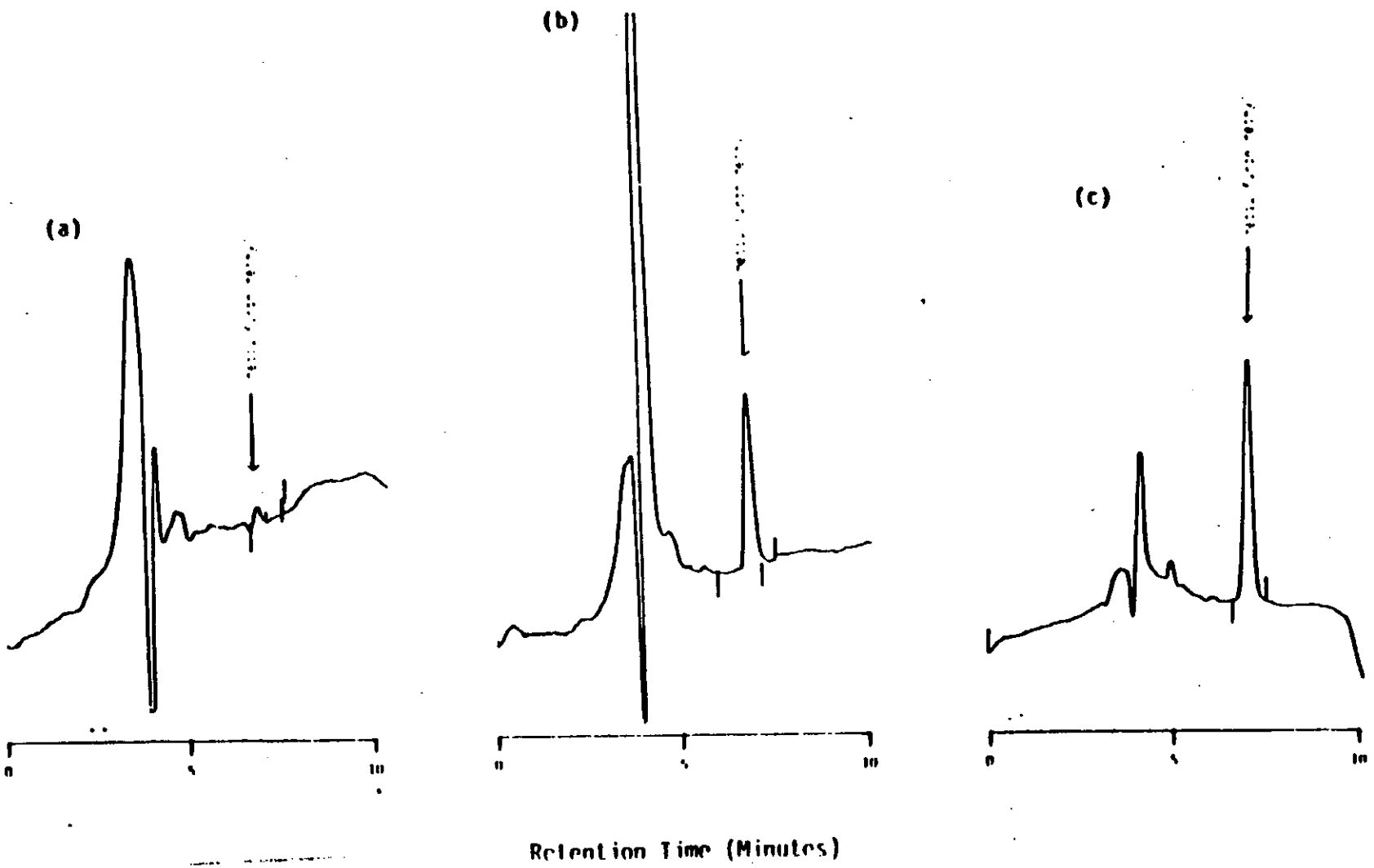



Figure 2. Residue levels of metsulfuron methyl in composited milk are plotted for the two cows treated at 20 ppm. Treatment of the cows was stopped after Day 28.

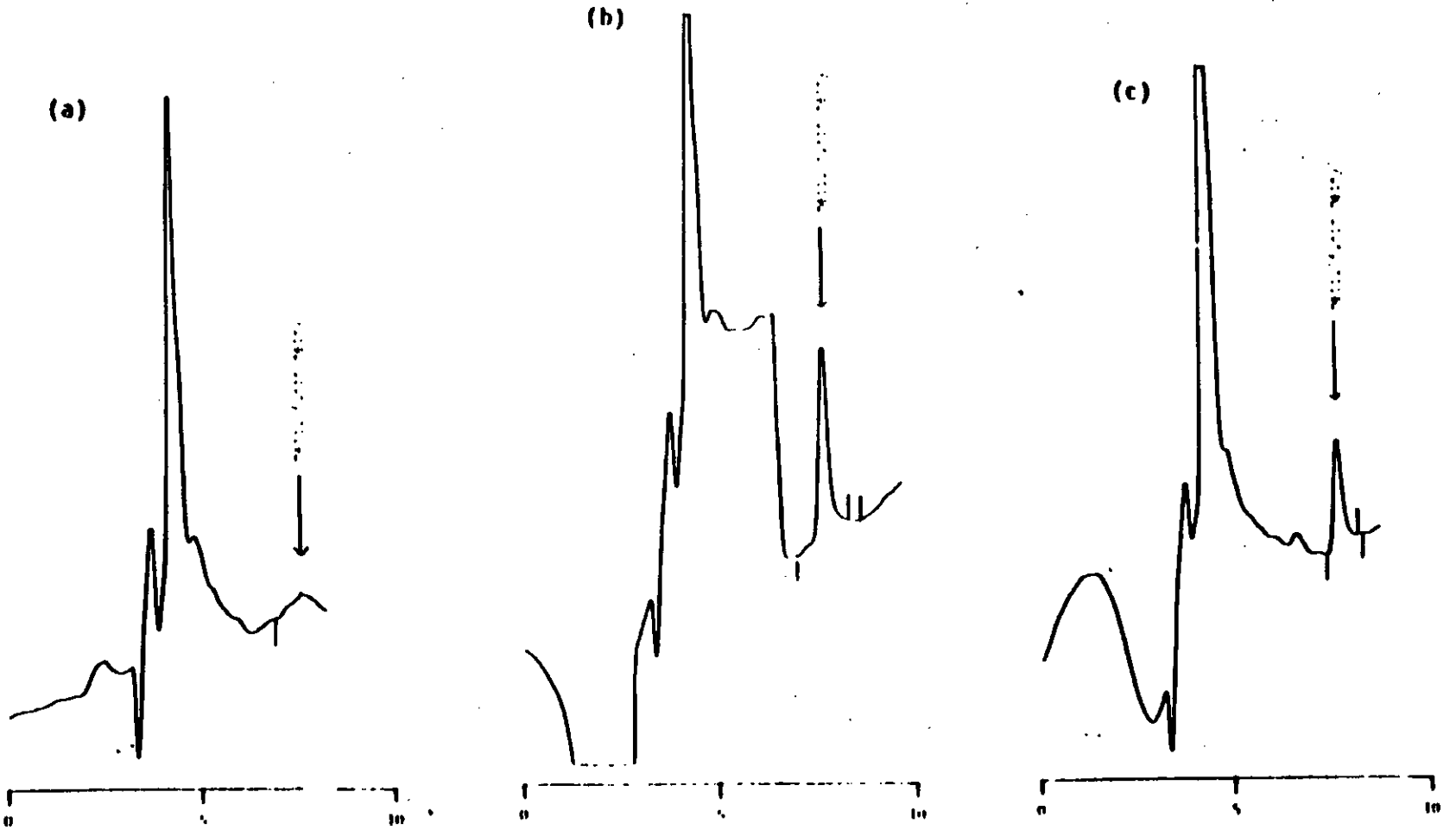



Figure 3. These are chromatograms of cream samples for (a) a control cow, (b) the same control fortified at 0.02 ppm with metsulfuron methyl, and (c) a 20 ppm treatment rate cow. The recovery for the fortified sample is 88% and the residue level for the treated cow is 11 ppb. All chromatograms are at an attentuation of 2.

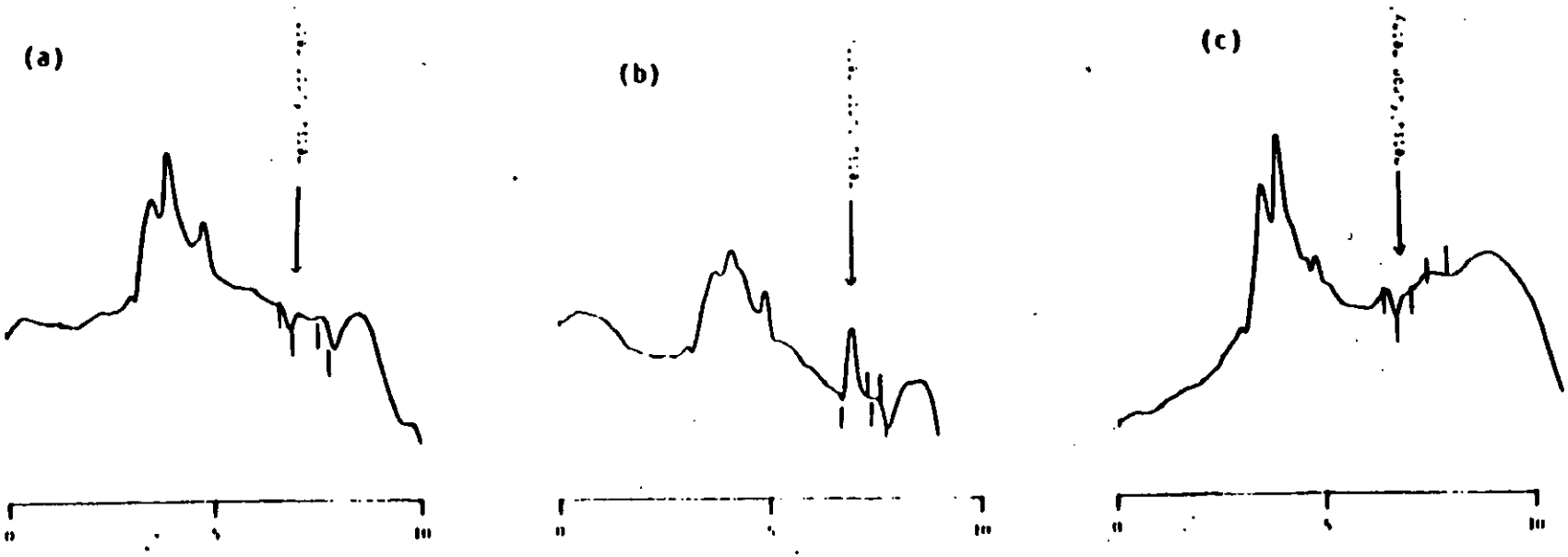



Figure 4. These are chromatograms of subcutaneous fat samples for (a) a control cow, (b) the same control fortified at 0.01 ppm with metsulfuron methyl, and (c) a 100 ppm treatment rate cow. The recovery for the fortified sample is 85%. All chromatograms are at an attentuation of 1.

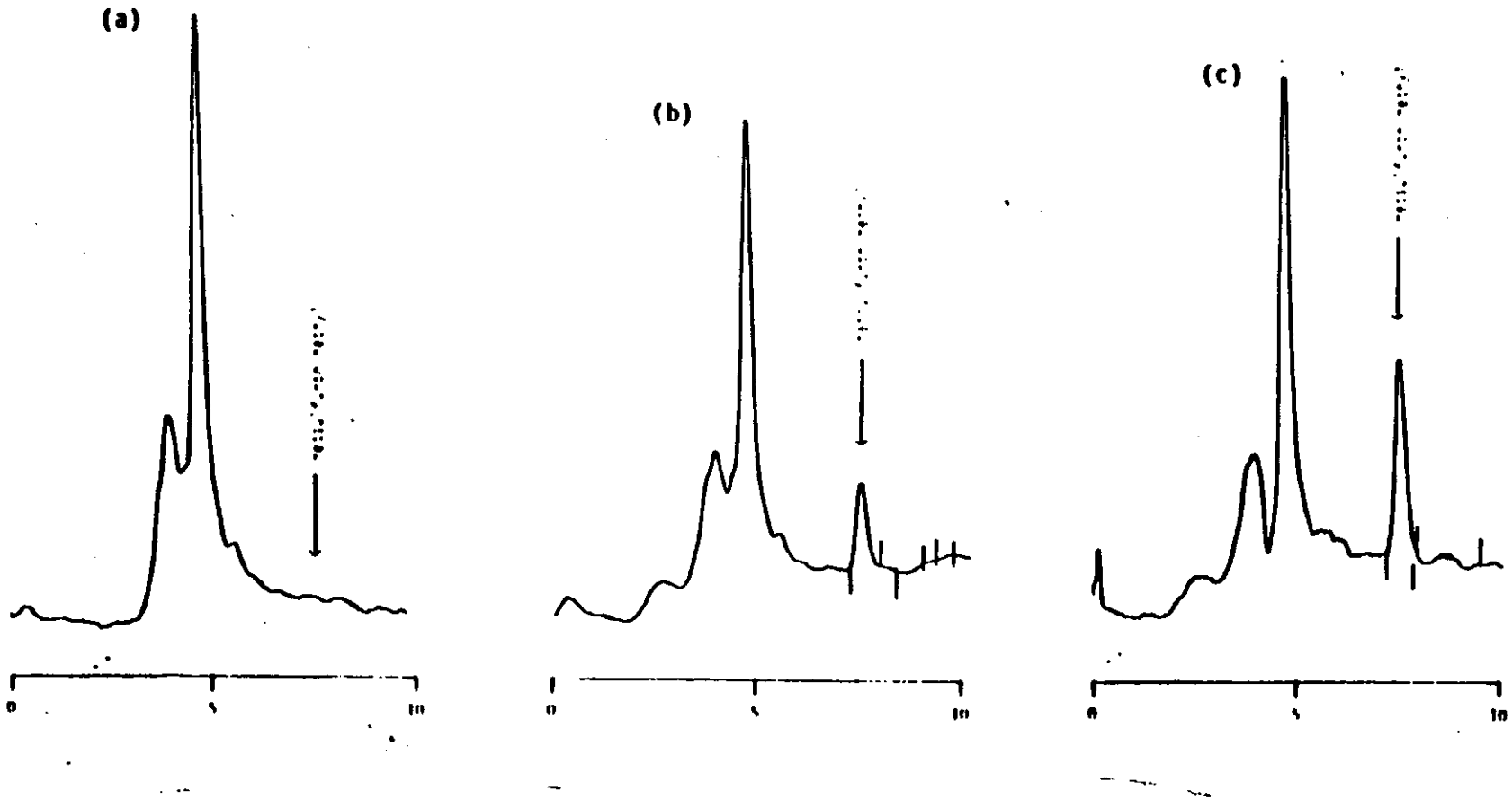



Figure 5. These are chromatograms of lean meat samples for (a) a control cow, (b) the same control fortified at 0.01 ppm with metsulfuron methyl, and (c) a 100 ppm treatment rate cow. The recovery for the fortified sample is 88% and the residue level for the treated cow is 20 ppb. All chromatograms are at an attentuation of 1.

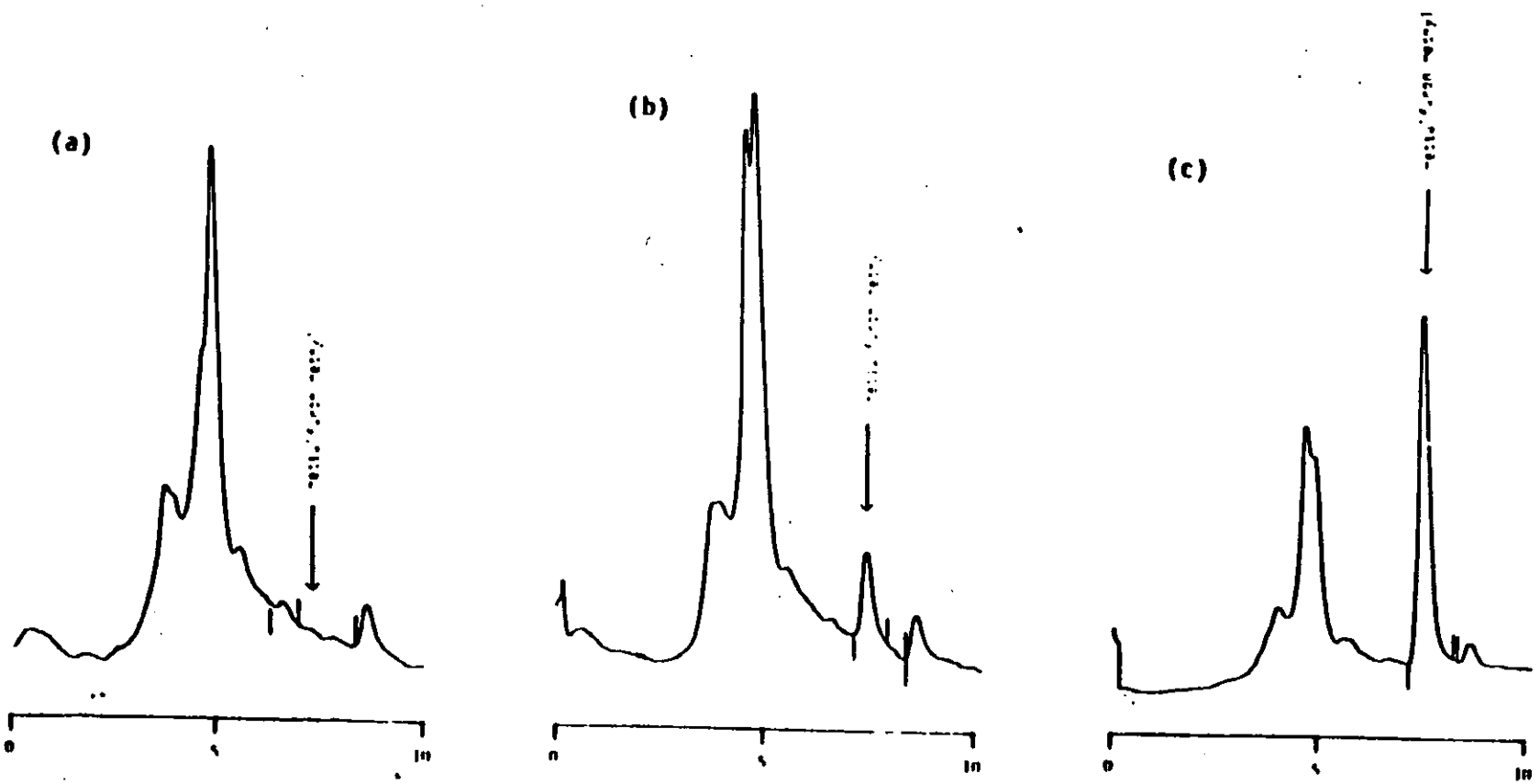



Figure 6. These are chromatograms of liver samples (a) a control cow, (b) the same control fortified at 0.01 ppm with metsulfuron methyl, and (c) a 100 ppm treatment rate cow. The recovery for the fortified sample is 93% and the residue level for the treated cow is 75 ppb. Chromatograms (a) and (b) are at an attentuation of 1 and chromatogram (c) is at 2.

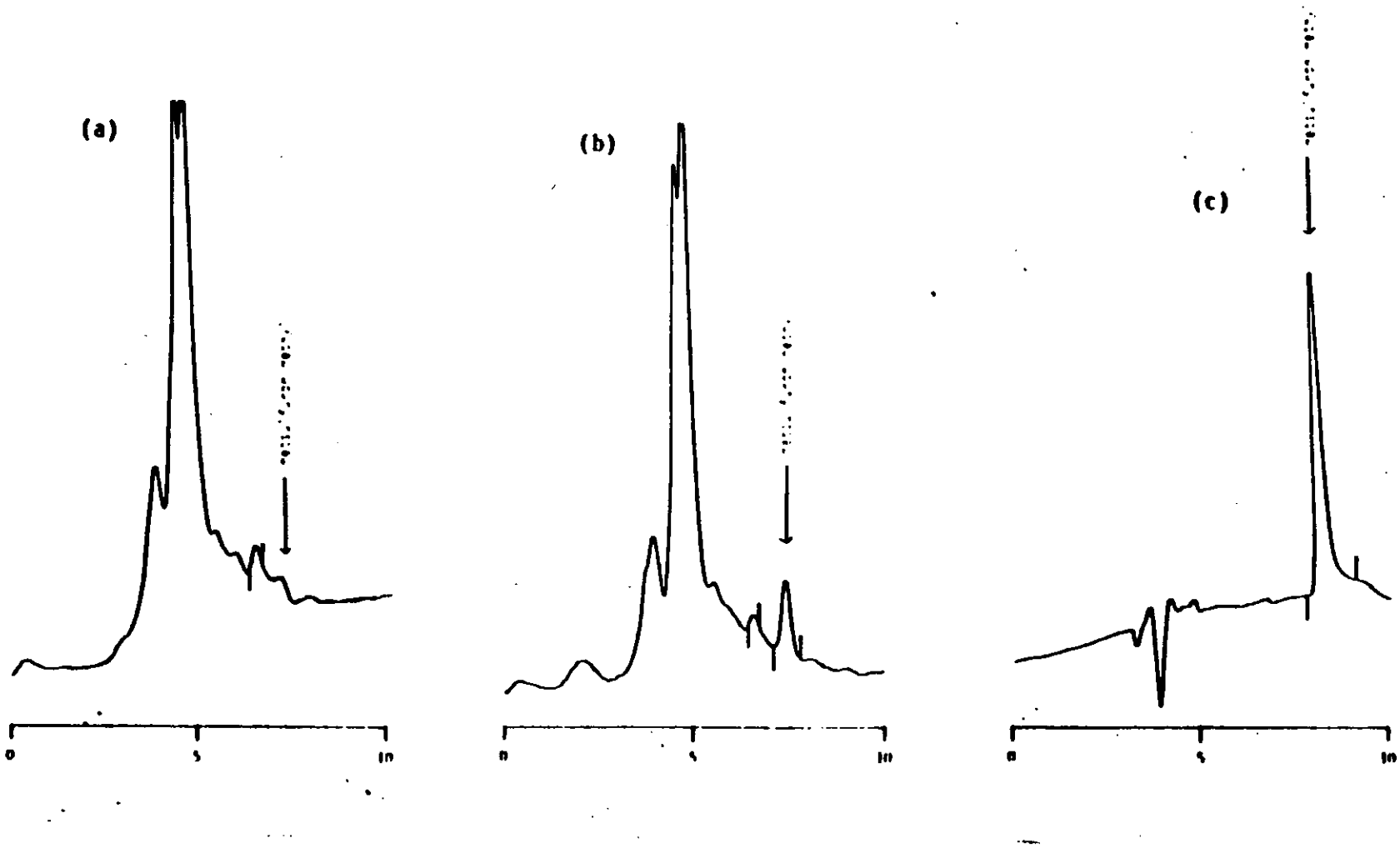



Figure 7. These are chromatograms of kidney samples for (a) a control cow, (b) the same control fortified at 0.01 ppm with metsulfuron methyl, and (c) a 100 ppm treatment rate cow. The recovery for the fortified sample is 80% and the residue level for the treated cow is 0.67 ppm. Chromatograms (a) and (b) are at an attentuation of 1 and chromatogram (c) is at 4 after the sample was diluted by 5.

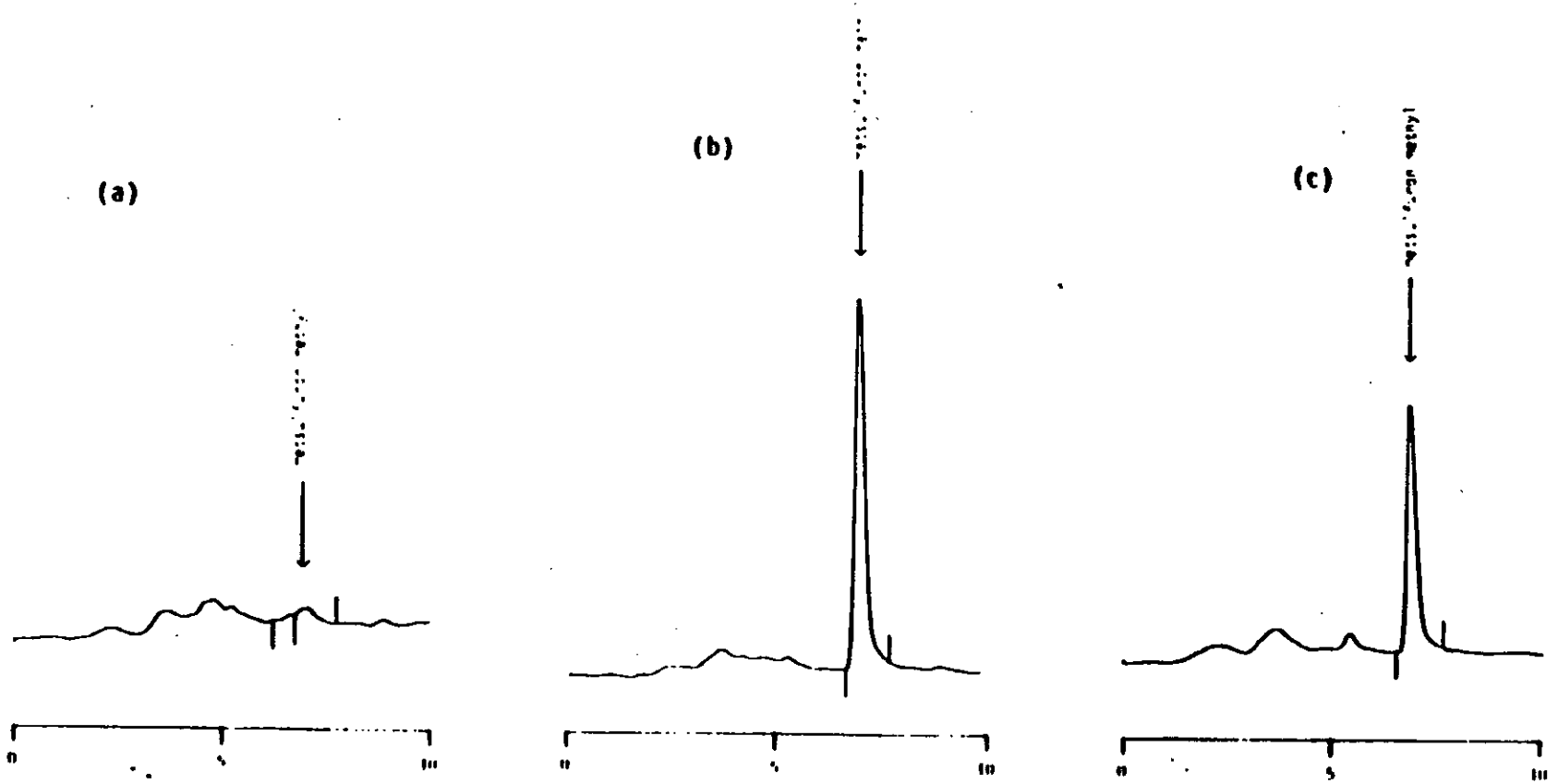



Figure 8. These are chromatograms of urine samples for (a) a control cow, (b) the same control fortified at 2.0 ppm with metsulfuron methyl, and (c) a 20 ppm treatment rate cow. The recovery for the fortified sample is 81% and the residue level for the treated cow is 11.0 ppm. Chromatogram (a) is at an attentuation of 1 and chromatograms (b) and (c) are at 2. The fortified control was diluted by a factor of 5 and the treated sample by a factor of 50.

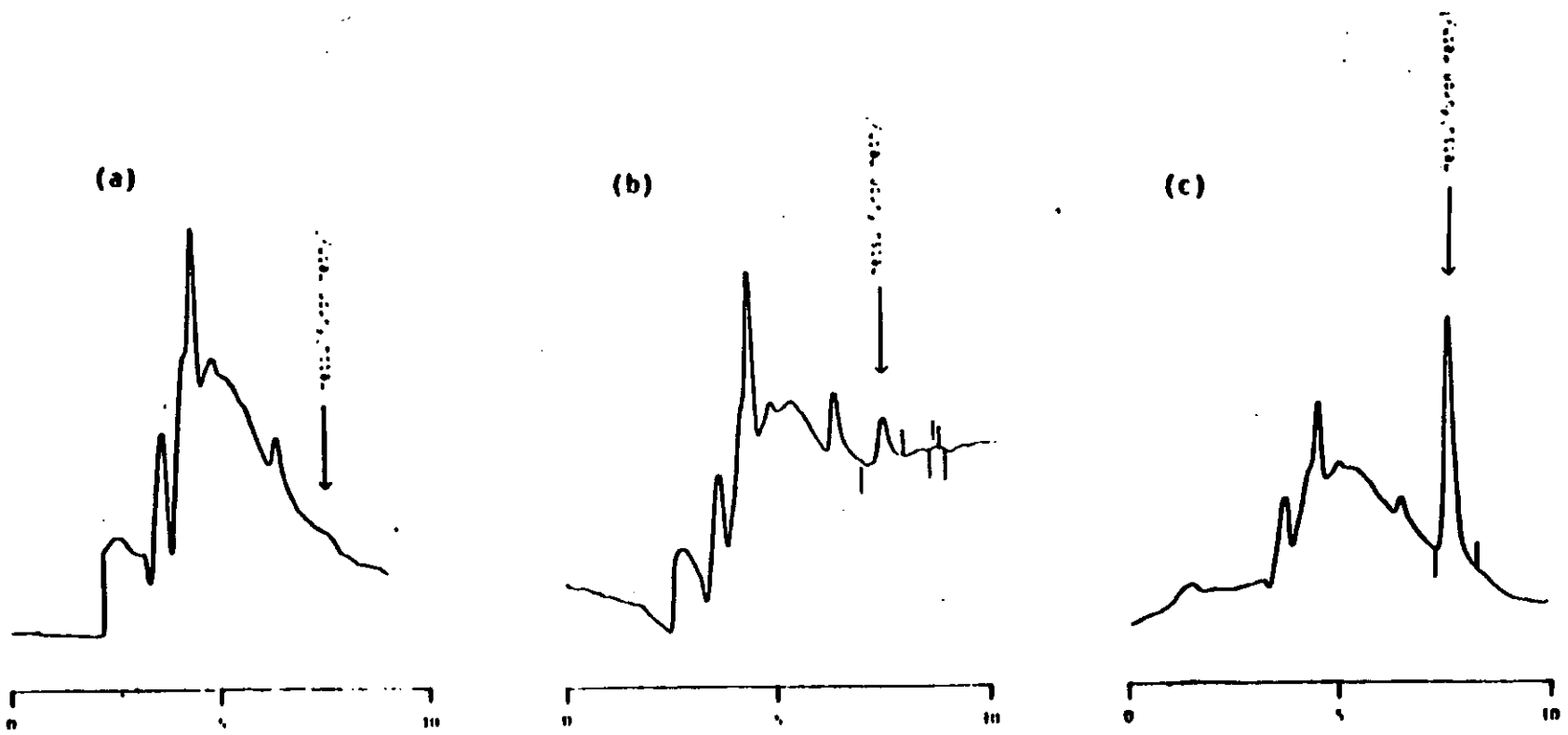



Figure 9. These are chromatograms of feces samples for (a) a control cow, (b) the same control fortified at 0.01 ppm with metsulfuron methyl, and (c) a 5 ppm treatment rate cow. The recovery for the fortified sample is 79% and the residue level for the treated cow is 85 ppb. Chromatograms (a) and (b) are at an attentuation of 2 and chromatogram (c) is at 4.